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Development of a genetic manipulation system for *Haemophilus parasuis*

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Abstract

Haemophilus parasuis is a member of the family *Pasteurellaceae* and an important respiratory-tract pathogen of swine, which is the etiological agent of Glässer's disease. Because no genetic manipulation system is available for *H. parasuis* so far, in vivo studies about the role of its genes involved in virulence are unfeasible. Here we demonstrate that *H. parasuis* has a cyclic AMP (cAMP)-dependent natural transformation system that enables the uptake of DNA in which the ACCGAACTC sequence signal must be present. After improving DNA transformation parameters, such as cAMP and DNA concentration and exposition time of the exogenous DNA, a knockout mutant of *H. parasuis* defective in the *thy* gene, encoding the thymidylate synthase enzyme, has been constructed. Data presented in this work open the possibility for the functional analysis of genes involved in the infectious process of this animal pathogen.

Keywords: *Haemophilus parasuis*; DNA transformation; Knockout mutants; Genetic manipulation

1. Introduction

Haemophilus parasuis is a Gram-negative bacterium belonging to the family *Pasteurellaceae*. This bacterial species is an important respiratory-tract swine pathogen that is the etiological agent of porcine poliserositis and arthritis syndrome, known as Glässer's disease (Kilian, 1976). *H. parasuis* infections produce significant mortality and morbidity in pig farms, giving rise to important economic losses in this industry. For this reason, the knowledge of virulence mechanisms of this organism, as well as its molecular biology, are relevant work-fields in progress. Recently, several putative virulence genes have

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been identified for *H. parasuis* (Hill et al., 2003), however, no systems for genetic manipulation are known, so, as a consequence, it has not been possible to obtain targeted mutants of *H. parasuis* so far.

It is known that several members of the family *Pasteurellaceae*, such as *H. influenzae* and *Actinobacillus actinomycetemcomitans*, present a natural transformation system (Albritton et al., 1984; Wang et al., 2002) if DNA contains a specific DNA-uptake signal sequence (USS) (Smith et al., 1999; Davidsen et al., 2004). In the present work, and taking advantage of this capacity of some *Pasteurellaceae* species, a transformation methodology for *H. parasuis* has been developed. Furthermore, this method has been used to construct an *H. parasuis* strain defective in *thy* gene that encodes the thymidylate synthase enzyme. This is the first description of a knockout mutant for *H. parasuis*.

2. Materials and methods

2.1. Bacterial strains, plasmids, growth conditions and genetic methods

Bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were grown in either Luria-Bertani rich medium (Sambrook et al., 1989) or AB minimal medium (Clark and Maaloe, 1967), with glucose (0.2%) and casamino acids (0.4%). *H. parasuis* was cultured on chocolated blood agar plates (BioMérieux, Inc.). Antibiotic concentrations employed for *E. coli* cultures have been described before (Cárdenas et al., 2001). For *H. parasuis*, streptomycin, kanamycin and trimethoprim were used at 150, 50 and 100 μg/ml, respectively. Plasmid and chromosomal DNA extractions were

Table 1
Bacterial strains and plasmids used in this work

	Relevant features	Source or reference
Bacterial strain		
E. coli		
DH5α	*supE4* Δ*lacU169* (Ø80 *lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Sambrook et al. (1989)
HB101	*supE4 hsdS20 recA13 ara-1 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	Clontech
KL742	λ$^-$ *thyA*748::Tn10 *rph-1 deo*-77	CGSC (Coli Genetic Stock Center)
MC1061 (λ*pir*)	*hsdR mcrB araD139* Δ(*araABC-leu*)7679 Δ*lacX*74 *gal1 galK rpsL thi*, lysogenized with λ*pir* bacteriophage	This laboratory
H. parasuis		
HP100	Parenteral strain, serotype 5, isolated from a naturally infected pig	I. Badiola (IRTA,Spain)
HP101	Str^R, spontaneous mutant of HP100	This work
HP102	As HP100, but *thy*	This work
Plasmid		
pBBR1MCS	A broad-host-range cloning vector, Cm^R Mob$^+$	BioTechniques
pGEM-T	PCR cloning vector, Ap^R	Promega
pGP704	Suicide and narrow-broad-host vector, Ap^R	Herrero et al. (1990)
pHRP309	A broad-host-range cloning vector, Gm^R Mob$^+$	Parales and Harwood (1993)
pRK2013	Mob$^+$ Tra$^+$ Km^R	Ditta et al. (1985)
pUA520	As pBBR1MCS, but Km^R	This laboratory
pUA658	As pGP704, but Gm^R	This laboratory
pUA1056	As pUA520, but carrying a 4-kb fragment containing the *H. parasuis thyA* gene	This work
pUA1057	As pHRP309, but carrying the *H. parasuis rps12* gene	This work
pUA1058	As pHRP309, but carrying the *H. parasuis rps12* gene without DNA USS sequence	This work
pUA1059	As pUA658, but carrying a 4-kb fragment containing the *H. parasuis thyA* gene interrupted by Km cassette	This work